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Limiting processes for perennial plant reintroduction to restore dry grasslands

Elise Buisson^{1,2}, Emmanuel Corcket^{3,4}, Thierry Dutoit¹

In restored grasslands of southern Europe, perennial plants remain highly underrepresented compared with the reference ecosystems. We tested various treatments to reintroduce common perennial plant species (*Brachypodium retusum*, Poaceae, and *Thymus vulgaris*, Lamiaceae), which are usually not or poorly reintroduced via soil and hay transfer. Treatments included microenvironmental manipulations (rock cover and plant interactions) and two grazing intensities. Target perennial species were transplanted in 2002 in the reference grassland ecosystem (intact grassland area used as a control) and in two abandoned fields. Survival was assessed in June 2003 and June 2004. Target species shoot and root biomass were measured in June 2004. Grazing greatly reduced the survival and biomass of both target species and its effects were reinforced by summer drought: plants that did not establish well enough during the autumn and spring did not survive summer. The restored rock cover had a mild positive effect, particularly on *B. retusum*. There were no negative or positive plant neighbor interactions in the steppe, while there was competition in both abandoned fields. Competition was particularly intense in the abandoned melon field, composed of a dense sward of annual grasses (*Bromus* sp.). In order to reintroduce perennial species to dry grasslands, the ideal combination of treatments is to exclude or reduce grazing during the first year to allow seedlings to establish and to recreate adequate microenvironmental conditions. Reducing competition from arable weeds may help but is not essential in such dry grasslands.

Key words: *Brachypodium retusum*, competition, grazing, Mediterranean steppe, plant interactions, rock, stone, *Thymus vulgaris*

Implications for Practice

- In order to reintroduce perennial species to Mediterranean dry grasslands, grazing should be excluded or reduced the first growing season after outplanting to allow seedlings to establish better. This was especially true for the studied chamaephyte *Thymus vulgaris*.
- Dense arable weed swards (*Bromus* sp.) significantly reduced the survival and growth of perennial species; the cost of removing arable weeds may however be prohibitive.
- In the Mediterranean climate, being well known for its strong interannual variations, improving microenvironmental conditions, such as restoring rock cover, may help to increase *Brachypodium retusum* (Poaceae) survival.

(3) degraded habitat quality, such as eutrophication (Fenn et al. 2010); and/or (4) competition through the presence of non-target species like arable weeds or exotic species (D'Antonio & Meyerson 2002). Grasslands around the Mediterranean Basin suffer the same types of degradation and their resilience is particularly low (Römermann et al. 2005; Buisson et al. 2006a; Coiffait-Gombault et al. 2012a). The restoration of such grasslands is of increasing interest, particularly in southeastern France where several large-scale projects have been implemented (Coiffait-Gombault et al. 2012b; Dutoit et al. 2013a, 2013b; Bulot et al. 2014; Jaunatre et al. 2014a, 2014b). In these projects, soil and hay transfer have led to positive results on plant species richness and composition, but some perennial species remain highly underrepresented in restored plots as

Introduction

Since the beginning of the twentieth century, both globally and in Europe, many pristine natural and seminatural grasslands have been lost or degraded by agricultural intensification: plowing, fertilization, drainage, liming, and overgrazing (White et al. 2000). Low resilience of grassland ecosystems after such disturbances can be due to (1) the lack of propagules in the seed bank and in the landscape (Perrow & Davy 2002; Römermann et al. 2005); (2) limited long-distance seed dispersal of herbaceous species (Perrow & Davy 2002; Buisson et al. 2006a);

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compared to the reference ecosystem (Coiffait-Gombault et al. 2012b; Jaunatre et al. 2014a, 2014b).

The aim of this study was to reintroduce two perennial plant species, *Thymus vulgaris* (Lamiaceae, chamaephyte) and *Brachypodium retusum* (Poaceae, hemicryptophyte) to degraded abandoned fields as a first step toward the restoration of Mediterranean grasslands. We chose these two perennial species because (1) they are common species in the western Mediterranean Basin, (2) they are dominant in the reference ecosystem (Buisson & Dutoit 2006), (3) they may improve habitat quality for other species (Buisson & Dutoit 2004; Coiffait-Gombault et al. 2012b) and (4) they are usually not reintroduced via soil and hay transfer (Coiffait-Gombault et al. 2011; Jaunatre et al. 2014a, 2014b). The main objective of this study was therefore to test the main limiting factors to reintroduce these perennial species by manipulating the microenvironment (rock cover) and biotic interactions (directly or via grazing).

Concerning the microenvironment treatment, we aimed to restore the original rock cover in abandoned fields to what it was in the reference grassland ecosystem before its cultivation. Rocks (>15 to 30 cm diameter) cover about 50% of the ground in the reference grassland ecosystem and had been removed for cultivation (Buisson & Dutoit 2006). Such rocks can provide physical nurse effects to plants, such as (1) enhanced soil moisture by increasing shade and thus reducing concomitant evaporation (Fowler 1988; Noy-Meir 2001; Elmarsdottir et al. 2003) or by increasing water condensation (Lahav & Steinberger 2001; Peters et al. 2008); (2) increased microbial activity under rocks due to increased soil moisture (Lahav & Steinberger 2001); (3) root protection from the heat, thus favoring perennials over annuals (Blumler 1992); (4) source of nutrients (Ugolini et al. 2001; Lopez et al. 2012); (5) wind sheltering, and (6) trapping fine sediments (Haussmann et al. 2010).

Concerning the biotic interactions, two treatments should be compared in our study: direct removal of plant neighbors and grazing. When competitive invasive or weedy plant species are found in a restoration site, their vigor and/or density has to be reduced because they may have a competitive advantage over target species (Perrow & Davy 2002; Gómez-Aparicio 2009). Some grassland restoration projects have used grazing in an effort to reduce competition of annual plant species over establishing perennials (Stahlheber & D'Antonio 2013), but this review was inconclusive overall because of site specificities. In this study, we tested whether grazing improved perennial species establishment in abandoned fields because our reference grassland ecosystem is traditionally grazed by sheep from February to June every year (Buisson & Dutoit 2006). In an effort to reduce competition of nontarget plant species, removal (cutting, mowing, herbicide application) of the aboveground biomass of standing vegetation is also used in restoration (Perrow & Davy 2002; Buisson et al. 2006b; Buisson et al. 2008). We therefore also tested the effects of hand removal of all plant neighbors in the vicinity of reintroduced perennials.

Plant interaction intensity can change with the level of abiotic constraint (Corcket et al. 2003) and plant strategies (Liancourt et al. 2005) in dry grasslands. Positive interactions between

plants are expected in harsh environments (Bertness & Callaway 1994) and may be an ecological force for ecosystem restoration (Brooker et al. 2008). In order to determine whether site agricultural history and soil fertility had an influence on perennial species establishment, we used three sites with various levels of nutrient availability: the reference grassland ecosystem (an intact grassland area, with low nutrient availability, considered as a control) and two abandoned fields (with higher fertility).

Altogether, the goal of this experiment was thus to test the effect of manipulating grazing (grazed or ungrazed), the microenvironment (rock cover), and plant neighbor interactions (neighbor removal or intact neighbors) on the survival and growth of two common Mediterranean perennial grassland species *T. vulgaris* and *B. retusum* in the reference grassland ecosystem (intact grassland area used as a control) and two abandoned fields.

Methods

Site Description

The studied area is located in the plain of La Crau, in southeastern France (43°33'E 4°50'N; 10 m a.s.l.). The reference grassland ecosystem composed of intact dry grasslands or pseudo-steppe of La Crau (phytosociological association: *Asphodeletum fistulosi* Molin. et Tallon—class: *Stipo-Trachynietea distachyae* Brullo), hereafter referred to as steppe, is species-rich with a unique set of common Mediterranean species that evolved with grazing as well as edaphic and climatic aridity (Buisson & Dutoit 2006). Parts of this area have been degraded through cultivation. The abiotic conditions of the fields are still altered 20–40 years after abandonment. In addition, the plant community is composed of arable weeds instead of steppe species although the traditional disturbance regime, grazing, was reestablished just after cultivation abandonment (Römermann et al. 2005; Sauguet 2013). Moreover, remnant intact patches of original steppe habitat have a limited role in supplying propagules to abandoned fields due to limited seed dispersal (Buisson & Dutoit 2004; Buisson et al. 2006a).

Soils (Haplic Cambisols) are less than 40 cm deep and are composed of 50% of 15–30 cm rocks (Buisson & Dutoit 2006). Typical for the Mediterranean climate, rainfall occurs mainly in autumn. Mean precipitation is 541 mm/year, varying between 350 and 800 mm depending on the year, and mean annual temperature is 15.3°C (data from 1997 to 2006; Wolff et al. 2013). Mean temperatures in 2003 were only slightly higher than during the previous year ($15.7 \pm 0.4^\circ\text{C}$ in 2003 vs. $15.4 \pm 0.3^\circ\text{C}$ in 2002). However, June temperatures were much higher in 2003 (T_{mean} in 2003: $25.5 \pm 0.4^\circ\text{C}$ vs. $22.5 \pm 0.5^\circ\text{C}$ from 2002 to 2004). The area received 683 mm of rainfall in 2003, which was less than that in 2002 (763 mm) (Istres weather station, 14 km from the study area). Shifting sheep flocks have been grazing the steppe and the cultivated sites before and after cultivation, from February to June (stocking rate of 4.6 animals ha yr⁻¹).

The first study site was a remnant patch of the reference grassland ecosystem or intact steppe (35 ha), representative of the remaining 10,500 ha, used as a control. The composition

of the intact steppe plant community was assessed by visually estimating all species % cover in forty-eight 40 cm × 40 cm quadrats. It was composed of 44 species and was dominated by the perennial grass *Brachypodium retusum* (20% cover) as well as *Dactylis glomerata* (6% cover), *Aegilops ovata* (5% cover), *Bellis sylvestris* (5% cover), and *Euphorbia cyparissias* (5% cover) (Buisson 2005; Table S1, Supporting Information). Plant community biomass and mean plant height were 179.6 g/m² and 3 cm, respectively.

The second site (a 12 ha abandoned cereal field) was intact steppe until 1960, cultivated with cereals between 1960 and 1966 and then abandoned. The disturbance involved chisel plowing, and some rock removal. The plant community (assessed in forty-eight 40 cm × 40 cm quadrats) at the time of this experiment, 38 years after cultivation abandonment, was composed of 49 species and was dominated by the annual grass *A. ovata* (29%) and the annual forb *Trifolium subterraneum* (11%) (Buisson 2005; Table S1). Plant community biomass and mean plant height were 178.8 g/m² and 2 cm, respectively. Soil samples taken in this site had significantly higher total N (0.22 mg/g of soil) than the two other sites (0.17 mg/g of soil) (Buisson 2005; Table S2).

The third site (a 5 ha abandoned melon field) was intact steppe until 1971, cultivated with melons between 1971 and 1975 and then abandoned. The disturbance involved moldboard plowing, watering, fertilizing, and rock removal (see Römermann et al. 2005 and Buisson & Dutoit 2004 for site history). The plant community (assessed in forty-eight 40 cm × 40 cm quadrats) at the time of this experiment, 29 years after cultivation abandonment was composed of 25 species, mainly annual grasses, such as *Bromus* sp. (26% cover), *Avena barbata* (10% cover), and *Vulpia ciliata* (6% cover) (Buisson 2005; Table S1). Plant community biomass and mean plant height were 112.8 g/m² and 4.8 cm, respectively. Soil concentrations of P₂O₅ and K₂O were significantly higher in this site (2.3 mg/100 g of soil and 17.3 mg/100 g of soil, respectively) than in the cereal field (1.3 mg/100 g of soil and 13.9 mg/100 g of soil, respectively) and the steppe (0.6 mg/100 g of soil and 9.5 mg/100 g of soil, respectively) (Buisson 2005; Table S2).

Experimental Design

Brachypodium retusum seeds were collected in June 2001 and *Thymus vulgaris* seeds were purchased locally. Seedlings were grown outdoors, in individual containers for 9 months, in 2/3 sand—1/3 soil from each site (steppe, abandoned cereal field, or abandoned melon field depending on where seedling were to be planted), watered as needed and not fertilized.

We used a split-split plot design where we manipulated grazing, rock cover, and competition (Fig. 1). At each site, we randomly set 12 experimental units in autumn 2002. Each unit was composed of two 3 m × 1.5 m plots. These two plots were located 3 m away from each other to avoid edge effects: one plot was fenced (ungrazed treatment) and the other was not fenced (grazed treatment). In the steppe, the effect of the presence of rocks was tested by removing rocks (>15 cm diameter) from

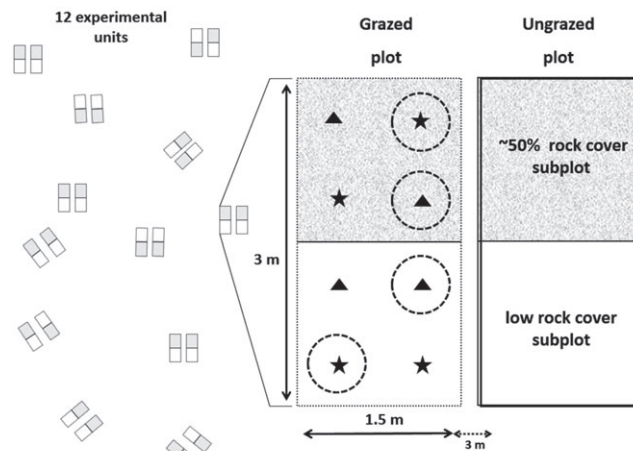


Figure 1. Experimental design layout. Each site had 12 experimental units composed of two 3 m × 1.5 m plots, one fenced plot (ungrazed treatment), and one nonfenced plot (grazed treatment). Each plot was split into two 1.5 m × 1.5 m subplots allocated to low rock cover (white) or 50% rock cover (shaded). Two *Thymus vulgaris* (stars) and two *Brachypodium retusum* (triangles) were planted in each subplot. Neighbor removal (dashed circle) was applied around one randomly chosen individual of each species.

one of each of the 1.5 m × 1.5 m subplots within a grazed treatment as well as within the ungrazed treatment. Conversely, in the abandoned fields, the effect of the presence of rocks was tested by restoring the 50% rock cover in one of each of the 1.5 m × 1.5 m subplots within a grazed treatment as well as within the ungrazed treatment. In October 2002, we transplanted two *T. vulgaris* and two *B. retusum* seedlings (randomly chosen among the seedlings grown on the soil corresponding to each planting site) in each subplot. The seedlings were watered only once when outplanted as plugs. To test the effect of the plant community (neighbors) on planted seedling, we hand-pulled all small seedlings and clipped all larger plants to the ground within a 25-cm diameter area surrounding one randomly chosen individual of each plant species in a subplot ($n = 12$ individuals/species/treatment combination × 3 sites; total number of plants of each species = 288). We removed neighbors in fall 2002, in spring 2003 and winter 2003–2004. Only seedlings that died within the first month were replaced.

Monitoring

Plant survival monitoring was carried out over a few contiguous days (1) in June 2003 and (2) in June 2004 (Fig. 2). In June 2004, we also collected shoot and root biomass of all outplanted *T. vulgaris* and *B. retusum* plants by sampling the same volume of soil (approximately 2 L) around each plant. Shoot and root were washed, dried (70°C) to a constant mass, and weighted.

Statistical Analyses

Analyses were performed using the program R (R Core Team 2015) and made use of the “coin” R package (Hothorn et al.

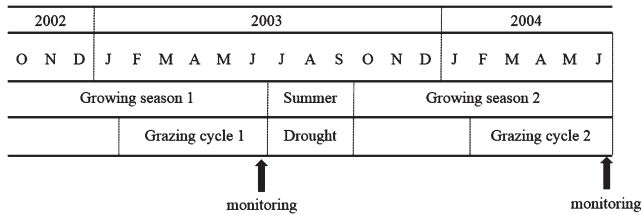


Figure 2. Plant survival monitoring was carried out over a few contiguous days (1) in June 2003, after one growing season and one grazing cycle, and before the 2003 summer drought; and (2) in June 2004, after two growing seasons, two grazing cycles and the particularly dry summer 2003 (Gobron et al. 2005). Planting occurred in October 2002.

2008). Permutation tests were used to analyze both survival and growth data. These tests (which are also called exact tests) are a modern and powerful alternative to tests based on statistics tables for which the assumptions are rarely verified in ecology (e.g. Mestre et al. 2013). Permutation tests are a type of statistical significance tests in which the population distribution is obtained by calculating the sample statistics under every possible permutation of the observed data points, and such tests are appropriate for small sample sizes.

For each species and for each site, the effects of the three treatments (starting by the interactions of the three treatments, then, if not significant by interactions of two treatments, and if not significant by treatments separately) on survival rate in 2003, survival rate in 2004, and shoot and root biomass were analyzed using permutation tests (Hothorn et al. 2008). When permutation tests were significant, multiple comparison tests were run and *p*-values recalculated with the Benjamini–Hochberg adjustment (Benjamini & Hochberg, 1995). The biomass data were unbalanced due to mortality, but the “coin” package does not require balanced designs (Hothorn et al. 2008).

Results

Transplant Survival in 2003

In 2003, survival of both species was relatively high, although that of *Brachypodium retusum* (84.7%) was lower than that of *Thymus vulgaris* (92.7%). Considered together, both species survival was significantly higher in the steppe (93.2%) than in the two other sites (melon field = 85.9%, cereal field = 87.0%).

The two species showed different survival patterns in the steppe: while treatments did not influence *B. retusum* (overall survival 92.7%), *T. vulgaris* had a higher survival rate either when not grazed or if grazed when neighbors were left intact (98.6 vs. 79.2%; Table 1).

The two species also showed different survival patterns in the former cultivated fields. In the melon field, treatments did not influence *B. retusum* (overall survival 83.3%), whereas grazing with restored rock cover reduced *T. vulgaris* survival (79.2 vs. 88.2%; Table 1). In the cereal field, treatment did not influence *T. vulgaris* (overall survival 95.8%), whereas grazing reduced *B. retusum* survival (68.8 vs. 87.5%; Table 1).

Table 1. Results of the permutation tests showing the effects of the treatments on the survival of transplanted *Brachypodium retusum* and *Thymus vulgaris* at each site in June 2003 and June 2004. The calculated statistic of the permutation tests is: (1) *z* when one explanatory variable (treatment) was tested or (2) max *T* when two or three explanatory variables (treatments) were tested.

	<i>B. retusum</i>	<i>T. vulgaris</i>
June 2003		
Steppe	n.s.	Grazing × Stones × Neighbors max <i>T</i> = 4.1, <i>p</i> = 0.017
Cereal field	Grazing <i>z</i> = −2.2, <i>p</i> = 0.046	n.s.
Melon field	n.s.	Grazing × Stones max <i>T</i> = 3.1, <i>p</i> = 0.015
June 2004		
Steppe	n.s.	Grazing <i>z</i> = −2.4, <i>p</i> = 0.032
Cereal field	Grazing × Stones × Neighbors max <i>T</i> = 3.6, <i>p</i> = 0.011	Grazing × Stones × Neighbors max <i>T</i> = 5.5, <i>p</i> < 0.001
Melon field	Grazing × Stones max <i>T</i> = 2.6, <i>p</i> = 0.042 Grazing × Neighbors max <i>T</i> = 2.6, <i>p</i> = 0.049 Stones × Neighbors max <i>T</i> = 3.1, <i>p</i> = 0.016	Grazing × Stones × Neighbors max <i>T</i> = 3.2, <i>p</i> = 0.008

Transplant Survival in 2004

In 2004, both species survival was low, although that of *B. retusum* (22.6%) was slightly lower than that of *T. vulgaris* (28.1%). Considered together, both species survival was significantly higher in the melon field (48.4%), intermediate in the cereal field (18.8%), and lower in the steppe (8.9%).

The two species showed different survival patterns in the steppe: while treatments did not influence *B. retusum* (overall survival 8.3%), grazing had a tremendous negative impact on *T. vulgaris* (2.1% vs. 16.7%; Table 1, Fig. 3).

Although the two species showed different survival patterns in the fields, they both were negatively impacted by grazing (e.g. in the melon field 25% of grazed *T. vulgaris* survived vs. 89.6% when not grazed). While rock cover restoration had significantly positive effects on *B. retusum* survival whatever the fields or the grazing treatment (e.g. in the cereal field 35.4% with rocks vs. 4.2% without rock; Fig. 3), it only had significantly positive effects on *T. vulgaris* survival when grazing was applied (in the melon field 33.3 vs. 16.1%; Table 1, Fig. 3). Plant neighbors mainly had a significant negative effect on *B. retusum* survival in the melon field (29.2% survived with neighbors vs. 50% without neighbors). In the melon field, rocks and neighbors significantly increased *T. vulgaris* survival when grazed, whereas they significantly decreased it when not grazed (Table 1, Fig. 3).

Transplant Biomass

Overall, *B. retusum* root biomass was much higher than that of *T. vulgaris* (*B. retusum* = 533.4 g and *T. vulgaris* = 2.1 g), whereas *T. vulgaris* shoot biomass was higher than that of *B. retusum* (*B. retusum* = 1.5 g and *T. vulgaris* = 11.0 g). Root and shoot

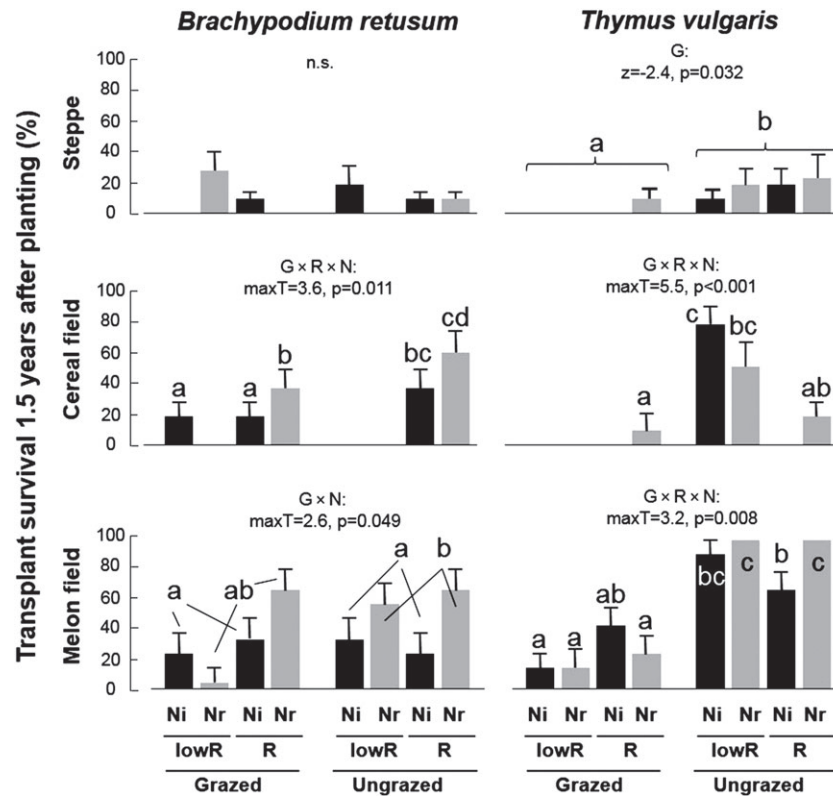


Figure 3. Mean (+SE) percent survival of transplanted *Brachypodium retusum* and *Thymus vulgaris* at each site in June 2004. Low R, rocks removed in the steppe or rocks intact in the fields; R, rocks intact in the steppe or rock cover restored in the fields; Ni, neighbors intact; Nr, neighbor removal. No bar for a particular treatment (e.g. *B. retusum*, in the steppe, grazed, low rock cover, with plant neighbors) means that all plants were dead 1.5 years after planting (0% survival; see Table 1 for all permutation test results). As we could not show the results of multiple comparison tests for all permutation tests, the tests selected to be presented are written above each of the six graphs (G, grazing; N, neighbors; n.s. = nonsignificant permutation tests; R, rocks). Within each of the graphs, bars showing similar letters did not differ significantly according to multiple comparison tests with Benjamini–Hochberg p adjustment. Treatments tested together were linked to common letters by plain lines.

biomass of both species were lower in the steppe than in the two fields. The potential effects of treatments on plant growth could not be analyzed because of low survival: for both species in the steppe and for *T. vulgaris* in the cereal field (Table 2).

Grazing had a negative effect on both species growth at all sites, but *T. vulgaris* was more sensitive to grazing than *B. retusum* (Fig. 4). Grazing significantly reduced *B. retusum* shoot and root biomass in the cereal field (e.g. root biomass = 370.7 g when grazed vs. 766.0 g when not grazed), whereas it significantly reduced *T. vulgaris* shoot and root biomass in the melon field (e.g. shoot biomass = 0.7 g when grazed vs. 13.0 g when not grazed) (Table 2; Fig. 4).

High rock cover had a positive effect on *B. retusum* growth only, stimulating both root and shoot biomass in the cereal field (e.g. root biomass = 327.4 g with high rock cover vs. 70.8 g with low rock cover) (Table 2; Fig. 4).

When significant, the effects of neighbors were negative suggesting the occurrence of competition. *Brachypodium retusum* shoot and root biomass were stimulated when plant neighbors were removed in cereal field, whereas both *B. retusum* (shoot biomass) and *T. vulgaris* (shoot and root biomass) were stimulated when plant neighbor were removed in the melon field (Table 2).

Table 2. Results of the permutation tests showing the effects of the treatments on the shoot and root biomass of transplanted *Brachypodium retusum* and *Thymus vulgaris* at each site in June 2004. For abbreviations and further explanation, see caption of Table 1.

	<i>B. retusum</i>	<i>T. vulgaris</i>
Shoot Biomass		
Steppe	n.s.	n.s.
Cereal field	Grazing × Stones × Neighbors max $T = 2.84$, $p = 0.03$	n.s.
Melon field	Neighbors $z = -2.04$, $p = 0.032$	Grazing × Stones max $T = 2.79$, $p = 0.023$ Grazing × Neighbors max $T = 3.69$, $p < 0.001$
Root biomass		
Steppe	n.s.	n.s.
Cereal field	Grazing × Stones × Neighbors max $T = 2.96$, $p = 0.027$	n.s.
Melon field	n.s.	Grazing × Stones max $T = 3.06$, $p = 0.005$ Grazing × Neighbors max $T = 3.63$, $p < 0.001$

Discussion

Establishment and survival in Mediterranean dry grasslands is a challenge even for stress-tolerant species: during the 2003

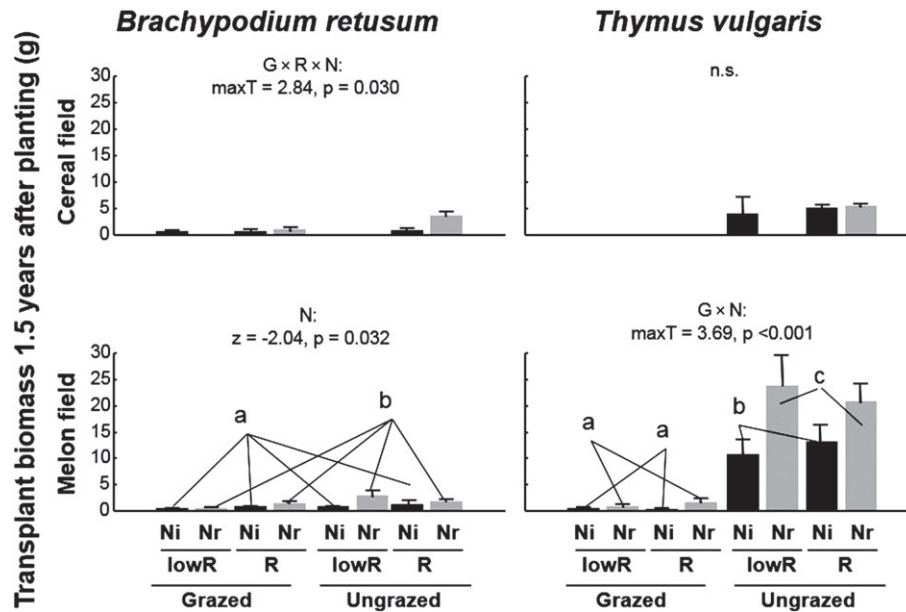


Figure 4. Mean (+SE) shoot biomass of transplanted *Brachypodium retusum* and *Thymus vulgaris* in abandoned fields in June 2004. low R, low rock cover; R, rock cover ~50%; Ni, neighbors intact; Nr, neighbor removal. No bar for a particular treatment (e.g. *T. vulgaris*, in the cereal field, grazed) means that all plants were dead 1.5 years after planting and no biomass could be weighed (see Table 2 for all permutation test results). For abbreviations and further explanation, see caption of Fig. 3.

summer drought, even some large greater than 10-year-old well-established *Thymus vulgaris* did not survive (E. Buisson 2003, IMBE—Université d’Avignon, personal observation). The Mediterranean climate is well known for its strong inter-annual variations and recruitment of perennial species likely occurs only under periodically favorable climatic conditions (i.e. in wetter/cooler years). The fact that this study was carried out over the particularly dry summer 2003 (Gobron et al. 2005) explains why so many plants died. Hence our plant biomass data can only be interpreted in the light of these high levels of mortality.

Differences Between Sites

In 2003, after the first grazing cycle and before the first summer drought, plant survival was slightly higher in the intact steppe than in the two other sites. This may be due to the fact that the intact steppe is composed at 20% by the perennial grass *Brachypodium retusum*, which is less palatable than the annual grasses and forbs of both abandoned fields. The sheep might actually eat less in the intact steppe although it was grazed by the same flock at the same pace. In 2004, after two growing seasons, two grazing cycles, and a particularly hot and dry 2003 summer (Gobron et al. 2005), root and shoot biomasses were lower in the steppe as a consequence of a poorer establishment probably due to lower soil nutrient availability (Römermann et al. 2005). This led to a final lower survival at this site and a higher survival in the fields.

Effects of Treatments in the Two Target Species

Grazing had a negative effect on survival and biomass of both species. This negative effect was lower on *B. retusum*

than on *T. vulgaris*. While the latter produces unpleasant secondary metabolites, *B. retusum* combines low palatability and digestibility (Cingolani et al. 2005), with high root production and tolerance to defoliation (i.e. hemicryptophyte with meristems at the soil surface; Briske 1996) so that plants of *B. retusum* were probably not harmed as much by the animals as plants of *T. vulgaris*. In the melon field, grazing was shown to have a negative effect on survival and on both shoot and root biomass of *T. vulgaris*. In this particular case, rocks and neighbors increased survival probably because (1) rocks partly protected plants against livestock grazing and (2) because the cover of the plant community in the melon field was composed of more than 40% of highly palatable annual grasses, which may have diluted the uptake on *T. vulgaris*.

In this study, in most cases, rocks had a positive effect on *B. retusum* survival and growth. Although we did not measure environmental parameters around and under rocks, Bourrelly (1984) showed that rocks create a microclimate and allow root biomass development in the steppe of La Crau. We can hypothesize that rocks allow *B. retusum* to protect roots from extreme heat during summer or/and to produce more roots (Ingelmo et al. 1994), which usually allow species to better cope with summer drought (Julander 1945). This would explain why rock nurse effect on survival of *B. retusum* was observed only after the first summer drought. Better root growth can be due to increased soil moisture (Fowler 1988; Lahav & Steinberger 2001; Noy-Meir 2001; Elmarsdottir et al. 2003; Peters et al. 2008). In addition, rocks might protect seedlings from total predation. Although sheep can turn pebbles over by kicking them to better reach their favorite plant food, they cannot displace larger rocks (E. Buisson 2003, IMBE—Université d’Avignon,

personal observation); this type of feeding behavior is common and has been observed in other rangelands (M. Meuret 2015, INRA, personal communication).

In the steppe, neighboring plants actually had a positive effect on *T. vulgaris* survival in 2003 when grazing was applied. Although 20% of the cover of plant community of the steppe is composed by *B. retusum*, more than 23% of it is composed of highly palatable species, such as *Dactylis glomerata* (5.6%), and annual species, such as *Brachypodium distachyon* (7.7%), *Bellis sylvestris* (5.1%), and *Aegilops ovata* (4.9%), which have been the sheep's main source of food (Meuret et al. 2013). As sheep are able to feed very selectively, the chamaephyte *T. vulgaris* was less preferred by the sheep and hence indirectly favored by neighboring plants.

When not grazed, neighbors in the melon field had a negative effect on both shoot and root biomass of both target species. The plant community of the melon field was composed of a dense sward of annual grasses (e.g. *Bromus* sp.) boosted by high nutrient concentrations. In other Mediterranean ecosystems, annual or biennial species have also been found to compete for resources with perennial species depending on their density and the season (Coiffait-Gombault 2011). Competition can be due to better soil water uptake (Clary et al. 2004; Moyes et al. 2005), light interception (Fehmi et al. 2004; Moyes et al. 2005), or litter interference (Lenz et al. 2003). In the ungrazed cereal field, *T. vulgaris* survival was lower when the rock cover was restored. After abandonment, this site was colonized by various legumes, such as *Trifolium subterraneum*, *T. stellatum*, *T. scabrum*, *Trigonella monspeliaca*, and *Medicago minima*. Rocks took the space that could have been colonized by these species and may have indirectly reduced the positive effect of these low-growing nitrogen-fixing neighbors on *T. vulgaris*.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. List of species composing the plant communities of the three sites: the steppe, the abandoned cereal field, and the abandoned melon field. The composition of the plant community was assessed by visually estimating all species % cover in one 40 cm × 40 cm quadrat in each subplot ($n = 48/\text{site}$). The numbers in the table are the mean % cover of each species at each site in the forty-eight 40 cm × 40 cm quadrats. Numbers in bold are the dominant species mentioned in the text.

Table S2. Soil pH, N, C, P_2O_5 , K_2O , CaO ($n = 24$); soil moisture (SM) ($n = 12$); and plant community characteristics ($n = 48$) at the three sites: steppe, abandoned cereal field, and abandoned melon field. In June 2004, 24 soil samples were randomly collected at each site, 12 inside and 12 outside the enclosures at each site and analyzed them for pH, Kjeldahl nitrogen, C, O.M., K_2O , P_2O_5 , MgO, and CaO. Soil moisture was measured after a dry period ($n = 12$) and after a rainy period ($n = 12$). Soil moisture was calculated using $SM = [(weight_{\text{humid}} - weight_{\text{dry}}) \times 100] / weight_{\text{humid}}$. At plant community level, aboveground biomass was assessed at each site by cutting all plants to the ground in four 50 × 50 cm quadrats in the vicinity of each experimental unit, in April 2004 (12 m² in 48 quadrats for each site) dried (70°C) to constant mass and weighted. Before cutting, mean plant height was assessed in each quadrat. Species richness was calculated from the plant community data taken in one 40 cm × 40 cm quadrat in each subplot ($n = 48/\text{site}$).